Remarks

Status of the Claims

Claims 1-22 have been canceled without prejudice or disclaimer of the subject matter contained therein. Claims 23-36 are new. Representative support for new claims 23-36 can be found in claims 1-22 as originally filed. The amendments to the claims do not add prohibited new matter.

Information Disclosure Statement

The Office Action objected to the presence of a reference in the IDS submitted June 5, 2009, for lack of an English translation. Applicants submit with this response a certified translation of the objected to reference.

Drawings

The Office Action objected to Figures 4 and 5 for the presence of shading that rendered text illegible. Applicants herein resubmit clean copies of Figures 4 and 5.

Sequence Compliance

The Office Action objected to the sequence listing for failing to list sequences presented in a drawing. Applicants submit with this paper an updated Sequence Listing, as well as a clean copy of Figure 5, to address the basis of this objection.

Rejection under 35 USC § 112, second paragraph

A. Claims 1-8, 10, 11, and 13-18 were rejected under 35 USC § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter regarded as the invention.

The Office Action alleged that the metes and bound of claims 1 and 2 are unclear. Without acquiescing to the merits of the rejection, claims 1 and 2 have been canceled without prejudice or disclaimer of the subject matter contained therein. New claims 23 and 24 correspond to claims 1 and 2. It is respectfully submitted that new claims 23 and 24 have addressed the bases for this rejection. It is therefore respectfully requested that this rejection be withdrawn.

B. Claims 3 and 8 were further rejected under 35 USC § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter regarded as the invention.

Claims 3 and 8 were rejected for reciting a broad range together with a narrow range that falls with the broad range. Claims 3 and 8 have been canceled without prejudice or disclaimer of the subject matter contained therein. New claims 25 and 30 correspond to claims 3 and 8. It is submitted that new claims 25 and 30 have addressed the basis for this rejection. It is therefore respectfully requested that this rejection be withdrawn.

C. Claims 5, 13, and 14 were further rejected under 35 USC § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter regarded as the invention.

Claims 5, 13, and 14, were rejected for lack of clarity through reciting the term "advantageously." Claims 5, 13, and 14 have been canceled without prejudice or disclaimer of the subject matter contained therein. New claim 27 corresponds to claim 5. It is submitted that new claim 27 has addressed the basis for this rejection. It is therefore respectfully requested that this rejection be withdrawn.

D. Claim 6 was further rejected under 35 USC § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter regarded as the invention.

Clam 6 was rejected for incorrectly referring to a previously introduced element in a prior claim. Claim 6 has been canceled without prejudice or disclaimer of the subject matter contained therein. New claim 28 corresponds to claim 6. It is submitted that new claim 28 has addressed the basis for this rejection. It is therefore respectfully requested that this rejection be withdrawn.

E. Claims 15 and 16 were further rejected under 35 USC § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter regarded as the invention.

Claims 15 and 16 were rejected for lack of clarity in reciting the term "standard immune repertoire." Claims 15 and 16 have been canceled without prejudice or disclaimer of the subject matter contained therein. New claims 31 and 32 correspond to claims 15 and 16. It is submitted that new claims 31 and 32 have addressed the basis for this rejection. It is therefore respectfully requested that this rejection be withdrawn.

Rejection under 35 USC § 103(a)

Claims 1-4, 6, 8, 17, and 18 were rejected under 35 USC §103(a) as allegedly being obvious over Pasqual, as evidenced by Biochemica and Krangel, in view of GenBank GI:21363121 ("GenBank"), Wu, and Arstila.

The claimed invention relates to a method for evaluating the rearrangement of genes, particularly with regard to evaluating the diversity of the TCR repertoire of an individual based on an obtained sample. The claimed invention amplifies the genomic DNA and provides for long fragments through primers, one of which is directed to a Vx gene and the other to a Jy gene. Vx and Jy are capable of rearrangement during V(D)J recombination. The amplified long fragments from genomic DNA are then separated by electrophoretic migration on a gel and then directly detected on the gel. The electrophoretic separation and in-gel detection allow one skilled in the art to identify a number of important rearrangements for Vx-Jz. The observed diversity in the gel is a combinatorial diversity, meaning that each band corresponds to a specific Vx-Jy rearrangement.

Pasqual teaches amplifying thymic genomic DNA from mice and evaluating the genetic rearrangements by Southern blotting. Pasqual teaches obtaining genomic DNA from the thymus of a sacrificed mouse. Pasqual does not teach obtaining genomic DNA from a blood or biopsy sample. Pasqual does not teach obtaining a human sample. The difference is significant.

Pasqual teaches obtaining a genomic DNA sample from a concentrated source. In practice, this step is not feasible for humans, as it would require the harvesting of the

thymus, a step which would entirely negate the purpose of the genetic analysis to begin with. Furthermore, one skilled in the art would not expect that a human sample would be able to yield a detectable product. It is the surprising and unexpected discovery that a human sample may be utilized and produce a product that is visible in a gel.

For example, in Figure 4 of the present specification, discrete and distinct bands of varying sizes are observed when the same pair of primers are utilized.

Furthermore, Applicants submit that a given band may correspond to different clones having the same Vx-Jy recombination, but will have diversity at the Vx-Jy junction. While the claimed method does not detect these particular types of diversity, such variances are not within the desired objective of the claimed invention. The separation conditions of the claimed invention allow one skilled in the art to discriminate between bands of variable sizes from between a hundred base pairs to several tens of thousands of base pairs.

Additionally, Pasqual teaches separating obtained PCR products on a 1.5% agarose gel and then Southern blotting with radio-labeled nucleotides, a process Pasqual admits as lacking in sensitivity (*see*, Pasqual at page 1170, left column, last paragraph). However, Pasqual regards radio-labeling to be the only means by which specificity can be determined (*see*, Pasqual at page 1165, left column, first full paragraph). It is of further note that radio-labels are distinctly disadvantageous in a clinical setting due to increased health risk and ease of contamination. Southern blotting is also disadvantageous in that it requires several days before a result is obtainable.

The authors of the reference of Pasqual are also inventors of the currently claimed invention and with this response are submitting further attestations that differentiate between their prior study on mouse thymus and the currently claimed invention. Applicants submit with this response a Declaration under 37 CFR 1.132 by Dr. Nicolas Pasqual. This Declaration highlights the shortcomings of the Pasqual reference, shortcomings that were not easily or readily overcome. For instance, the Declaration of Dr. Pasqual points to the length of time, the use of radioactive substances, and the quantification bias that the Pasqual reference is subject to. Dr. Pasqual further states that at the time of the Pasqual reference, observation of the rearrangements directly on the gel was not possible. The Declaration of Dr. Pasqual further states that at the time of the

Pasqual reference observation of the rearrangements was not possible in the gel, and could only be achieved by radio-labeling in a Southern blot (*see* Exhibit A attached to the Declaration of Dr. Pasqual). Figure 1A of Exhibit A demonstrates that only smears were observable in the gel (not a coloration problem as evidenced by the clarity of the size markers) and that distinct bands only appeared after radio-labeling in a Southern blot (Figure 2A of Exhibit A). Similarly, Exhibit B of the Declaration of Dr. Pasqual shows comparable results wherein Figures 1B and 2B smears are obtained, but in Figure 3B discernable bands are observed directly in the gel utilizing the steps of the claimed invention.

Accordingly, by following the steps of the claimed method, one is able to unexpectedly improve the readability of the gel as compared to what was possible based on the reference of Pasqual. The significant differences between the Pasqual reference and the claimed invention are the use of a human biological sample, the length of the elongation step during amplification, and the detection step being available directly in the gel, without transfer to a membrane or the use of radio-labeled probes, all of which enable one skilled in the art to visualize many rearrangements per line directly in the gel. Exhibit C of the Declaration of Dr. Pasqual further illustrates that the claimed invention works.

With respect to the other cited references, Krangel provides only a review of mechanism of recombination for RSS and RAG proteins. Krangel does not teach a method to determine of Vx-Jy recombination in human genomic DNA by in gel analysis of a long PCR product obtained through a primer to Vx and a primer to Jy.

Biochemica teaches only the basic principles for Expand High Fidelity PCR. Biochemica does not teach a method to determine of Vx-Jy recombination in human genomic DNA by in gel analysis of a long PCR product obtained through a primer to Vx and a primer to Jy.

GenBank teaches to sequence of human TCRAD. GenBank does not teach a method to determine of Vx-Jy recombination in human genomic DNA by in gel analysis of a long PCR product obtained through a primer to Vx and a primer to Jy.

Wu teaches parameters to consider in adjusting for optimizing PCR. Wu does not teach a method to determine of Vx-Jy recombination in human genomic DNA by in gel analysis of a long PCR product obtained through a primer to Vx and a primer to Jy.

Arstila teach amplification of human cDNA, not gDNA, for T cell receptor alpha-beta diversity through analysis of the CDR3 region. The drawbacks of the method of Artsila are also discussed on p[age 10 the present specification. Arstila does not teach a method to determine of Vx-Jy recombination in human genomic DNA by in gel analysis of a long PCR product obtained through a primer to Vx and a primer to Jy.

Accordingly, one skilled in the art could not arrive at the claimed invention through any combination of the cited references. No other cited reference provides the necessary information to overcome the deficiencies of Pasqual to arrive at the claimed invention. As evidenced by the working examples and the Declaration of Dr. Pasqual, the claimed invention provides a significant improvement in the art over the reference of Pasqual as the claimed invention provides for visualization of a larger number of rearrangements in a single visualization step without the need for radio-labels and membrane transfers.

Moreover, it is only though the combination of these improvements over the reference of Pasqual that the claimed invention is possible. For example, simply increasing the elongation time would still yield intense smears. Similarly, transferring a human sample to a membrane for Southern blotting is susceptible to a failure of detection given the lack of sensitivity.

Therefore, it is only through combining the specific features of the use of a human biological sample, the length of the elongation step during amplification, and the detection step being available directly in the gel, without transfer to a membrane or the use of radio-labeled probes, that the claimed invention is possible. None of the cited references disclose or suggest these features. Accordingly, it is believed that the claimed invention is novel and non-obvious over the cited references. It is therefore respectfully requested that this rejection be withdrawn.

If there are any additional fees due in connection with the filing of this response, please charge the fees to our Deposit Account No. 50-0310. If a fee is required for an

extension of time under 37 C.F.R. §1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,
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